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Pharmacokinetics and Fibrin Specificity of Alteplase During Accelerated Infusions in Acute Myocardial Infarction

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Pharmacokinetics and fibrin specificity of alteplase (recombinant tissue-type plasminogen activator) were determined in 10 patients with acute myocardial infarction undergoing an accelerated infusion regimen during the alteplase/anistreplase patency study (TAPS). Fifteen milligrams of alteplase was administered as an intravenous bolus injection, followed by infusions of 50 mg over 30 min and 35 mg over a further 60 min.

Mean steady state plasma concentrations of alteplase during the initial 30 min were $3.2 \pm 0.84 \mu\text{g/ml}$, measured immunochemically, and $2.1 \pm 0.23 \mu\text{g/ml}$, measured using a functional activity assay. These values were 45% and 51% higher, respectively, than those during the standard infusion schedule ($p < 0.01$). However, the predominant plasma half-life determined by model fitting

based on either assay (3.3 to 3.5 min) was unaltered compared with the standard regimen. Maximal concentrations of fibrin and fibrinogen degradation products were 5.1 ± 2.2 and $1.9 \pm 1.1 \mu\text{g/ml}$, respectively. Plasminogen decreased to 70% and alpha₂-antiplasmin to 35% of values before infusion.

The results indicate that 1) improved coronary patency rates during "front-loaded" infusions can be rationalized in terms of higher plasma concentrations of both free and immunoreactive alteplase, 2) kinetic variables are comparable with those of other dosing strategies, and 3) fibrin specificity is not diminished relative to that of the standard infusion regimen.

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Timely thrombolytic therapy using alteplase (recombinant tissue-type plasminogen activator) during acute myocardial infarction achieves rapid patency of the infarct-related coronary artery and results in improved survival (1). Alteplase is the only thrombolytic agent in clinical use that demonstrates significant clot selectivity (2).

The currently recommended alteplase dose in acute myocardial infarction is 100 mg over 3 h, consisting of 10 mg as an initial intravenous bolus injection, followed by infusions of 50 mg in the 1st h and 20 mg/h during the 2nd and 3rd h (prescribing information, Boehringer Ingelheim GmbH and Genentech). With this regimen, an angiographically documented coronary patency rate of 71% after 90 min has been reported (3). More recently, however, several studies (4) have been performed with the aim of optimizing further the dosing of alteplase. In particular, Neuhaus et al. (5) achieved a very high coronary patency rate of 91% at 90 min using an accelerated "front-loaded" regimen (6). With this dosing schedule, 100 mg was given in 90 min, comprising an

initial 15-mg intravenous bolus injection, followed by infusions of 50 mg in 30 min and 35 mg in 60 min.

There have been few systematic pharmacokinetic investigations of the currently marketed preparation of alteplase in myocardial infarction (7). We recently reported studies using the standard infusion regimen (8) and a single 50-mg bolus injection (9). The aims of the present study were to measure alteplase plasma concentrations and hemostasis variables in patients undergoing the accelerated dosing schedule of Neuhaus et al. (5), and to assess whether the increased efficacy of this regimen can be rationalized in pharmacokinetic terms. The patients were a subset of a recent cooperative randomized trial comparing alteplase and anistreplase with respect to early coronary artery patency (10).

Methods

Study patients. From April 1989 to May 1990, 10 patients (9 men and 1 woman) who were participating at the Göttingen University Clinic center in the collaborative alteplase/anistreplase patency study (TAPS) (10) were selected for additional pharmacokinetic analysis. Inclusion and exclusion criteria were as previously described (10,11). Patients randomized to alteplase at this center were sequentially assigned to the pharmacokinetic study provided that they were able, in the clinician's assessment, to tolerate the additional blood sampling procedures. The study protocol

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was approved by the Review Board of the hospital, and the patients gave informed consent, which was written or witnessed. The essential baseline characteristics of the patients were the following: age 56 ± 8 years (range 46 to 69), body weight 79 ± 12 kg (range 54 to 93), mean time between onset of symptoms and start of alteplase therapy 2.5 ± 0.9 h (range 1.3 to 4).

Administration of alteplase, coronary angiography. Alteplase (Actilyse, Dr. Karl Thomae GmbH) was administered intravenously into a forearm vein of each patient by means of a controlled-rate infusion pump. Fifteen milligrams was infused in exactly 2 min to simulate a bolus injection, followed by 50 mg in 30 min and 35 mg in 60 min. All patients received intravenous heparin; the dose was a 5,000-IU bolus injection before administration of alteplase was started, followed by heparin infusion at a rate that was adjusted to maintain the thrombin time 2.5 times higher than the upper limit of normal. Coronary angiography was an essential feature of the TAPS study protocol and was performed 60 and 90 min after the start of alteplase infusion. Vessels were considered patent if their angiograms corresponded to grade 2 or 3 of the Thrombolysis in Myocardial Infarction (TIMI) classification (3).

Blood sampling and assays for alteplase. At baseline and at 15 additional time points during and after alteplase infusions, two 4-ml blood samples were taken from an antecubital vein in the forearm contralateral to the infusion site, using disodium ethylenediamine tetraacetic acid (EDTA) as anticoagulant. The latest sampling time was 24 h after the initiation of alteplase infusions. To the 1st 4-ml sample, D-Phe-Pro-Arg-CH₂Cl (Calbiochem, Frankfurt) was added to a final concentration of $2 \mu\text{M}$ to inhibit binding of alteplase to plasma proteins before measurement using enzyme-linked immunosorbent assay (ELISA) (12). The second sample, without added inhibitor, was used to measure alteplase functional activity. Plasma was prepared within 15 min, frozen and kept at -20°C until analysis. Alteplase was measured both as antigen with use of a two-site assay (ELISA) and as functional activity with use of a chromogenic assay that included glu-plasminogen, cyanogen bromide fragments of fibrinogen and the substrate S-2251 (H-Val-Leu-Lys-para-nitroanilide). Both assay procedures have been previously described in detail (13,14). The detection limits of these assays for alteplase in plasma samples were 0.3 ng/ml (ELISA) and 3 ng/ml (chromogenic activity).

Assays for hemostasis variables. At baseline, at 2 or 4 h and at 24 h after the start of alteplase infusions, further plasma samples were obtained for measurement of hemostasis variables. Plasminogen and alpha₂-antiplasmin were determined amidolytically with use of chromogenic substrates and fibrinogen was determined with use of a clotting rate assay, as described previously (13,14). Degradation products of cross-linked fibrin and of fibrinogen were measured with use of specific monoclonal antibody-based ELISA methods (15) that are available commercially as kits (Fibrinostika, Organon Teknika, The Netherlands). The detection limit of

both ELISA methods was $0.01 \mu\text{g/ml}$, and the upper limits of the normal ranges for healthy persons, as specified by the manufacturer, were $0.31 \mu\text{g/ml}$ for fibrin degradation products and $0.25 \mu\text{g/ml}$ for fibrinogen degradation products.

Data analysis. Alteplase plasma concentration-time profiles were fitted for each patient by means of the program TOPFIT (16). Baseline alteplase concentrations, when measurable, were subtracted from all subsequent concentrations before fitting. The two-compartment model depicted in the inset to Figure 2 was used, which incorporated elimination from the central (plasma) compartment, reversible distribution into one peripheral (tissue) compartment and consecutive zero-order drug inputs (infusions) into the central compartment. The following variables were calculated from the model with standard formulas (17): maximal plasma concentration after the 15-mg bolus injection and steady state concentrations during the 50- and 35-mg infusions, respectively (Fig. 2); total clearance, plasma half-lives and their corresponding partial areas under the curve, and the volumes of distribution of the central compartment and at steady state.

Results are expressed as mean values \pm SD unless otherwise stated. The activity/antigen ratio of alteplase was determined for each patient by preparing individual plots of activity versus antigen over all data points of the plasma concentration-time profile and determining the slope of the linear regression line. Other comparisons were performed by using *t* tests for paired or unpaired samples, as appropriate; *p* values < 0.05 were considered significant.

Results

Clinical course. The treatment with alteplase was tolerated well by all patients, and no adverse events such as bleeding occurred. The coronary angiograms obtained 90 min after the start of alteplase infusions revealed a patent infarct-related artery in 8 of the 10 patients. Comparison of demographic, pharmacokinetic and hemostasis variables revealed no significant differences between the patient subgroups that exhibited a patent or a nonpatent vessel.

Pharmacokinetics. The time course of alteplase plasma concentrations in the patients, measured as antigen and functional activity, is shown in Figure 1. An example of a pharmacokinetic fit of the data from one patient is given in Figure 2, and the mean model-derived variables are summarized in Table 1. Alteplase antigen concentrations in plasma reached a maximum of $4 \mu\text{g/ml}$ after the 15 mg bolus injection, maintained an initial steady state value of $3.2 \mu\text{g/ml}$ during the first infusion of 50 mg over 30 min and decreased to a second steady state of $1.08 \mu\text{g/ml}$ during the subsequent infusion of 35 mg over 60 min.

Steady state plasma concentrations based on alteplase functional activity were approximately 30% lower than antigen concentrations, and therefore the clearance calculated from functional activity was correspondingly higher. These differences were statistically significant (Table 1). Also, 24 h

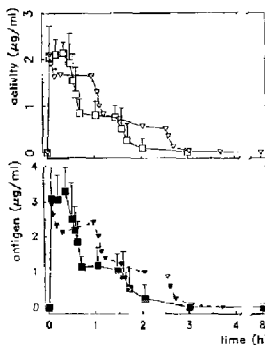


Figure 1. Time course of alteplase plasma concentrations after accelerated infusions (squares). Alteplase was measured both as antigen concentration (closed symbols, lower panel) and functional activity (open symbols, upper panel). Data points are shown as mean values \pm SD. For comparison, mean profiles (SD omitted for clarity) are also shown during the standard infusion schedule (triangles), for which the antigen data are derived from reference 8 and the activity data are new.

after the start of infusions, alteplase antigen concentrations were significantly elevated over baseline values, whereas functional activity at both these time points was below the assay detection limit.

The time profiles of alteplase antigen and functional activity during alteplase therapy were, however, closely similar. In particular, alteplase concentrations measured in both assays declined biphasically after termination of the infusions. A rapid alpha-phase with a half-life of 3.3 to 3.5 min was followed by a longer beta-phase with a half-life of 72 to 88 min. The alpha-phase of alteplase disposition was dominant, because it accounted for 85% to 88% of the area under the curve. Half-lives and partial areas under the curve derived from functional activity were not significantly different from those based on antigen concentration; the same was true for the volumes of distribution of the central compartment and at steady state.

Comparison with standard infusion kinetics. Alteplase plasma concentration profiles from the standard infusion regimen are included for comparison in Figure 1. The antigen values were taken from reference 8; however, functional activity measurements from the patients of that study are reported for the first time here. Initial steady state values listed in Table 1 during accelerated infusions were 45% and 51% higher in terms of antigen concentration and activity, respectively, than during the standard infusion regimen ($p < 0.01$). Second steady state concentrations were 16% higher in terms of antigen ($p = NS$) and 33% higher for activity

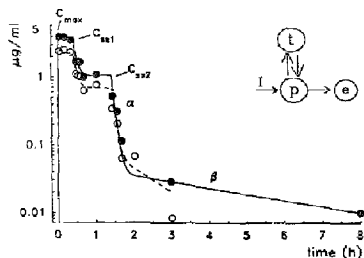


Figure 2. Examples of model fits of alteplase antigen (closed symbols, solid curve) and functional activity (open symbols, broken curve) in plasma samples from one patient (a 52-year old woman); α and β denote the two phases of disposition observed after termination of the infusions. Inset: two-compartment model used for fitting, with the compartments p (plasma), t (tissue), e (elimination). The leftmost arrow represents the infusion input (I); the remaining arrows are first-order transfers of alteplase between the compartments. C_{max} = maximal plasma concentration after 15-mg bolus injection; C_{ss1} and C_{ss2} steady state concentrations during the 50- and 35-mg infusions, respectively.

($p < 0.01$). There were no significant differences between the two regimens with respect to the dominant (alpha) plasma half-life and the volumes of distribution of the central compartment and at steady state; however, total plasma clearance was significantly more rapid compared with that of the standard regimen (572 vs. 380 ml/min based on antigen; 829 vs. 661 ml/min for activity; $p < 0.001$).

Table 1. Alteplase Pharmacokinetic Variables After Accelerated Dosing in 10 Patients With Acute Myocardial Infarction

	Antigen	Functional Activity	p Value
Baseline concentration (ng/ml)	6.1 ± 5.3	*	
Concentration at 24 h (ng/ml)	12.4 ± 6	*	0.04†
C_{max} (µg/ml)	4 ± 1	2.9 ± 0.81	0.02
C_{ss1} (µg/ml)	3.2 ± 0.84	2.1 ± 0.23	0.006
C_{ss2} (µg/ml)	1.08 ± 0.31	0.76 ± 0.17	0.002
CL (ml/min)	572 ± 132	829 ± 183	0.001
$t_{1/2\alpha}$ (min)	3.5 ± 1.4	3.1 ± 0.9	NS
$t_{1/2\beta}$ (min)	72 ± 68	88 ± 68	NS
$AUC_{0-\infty}$ (%)	85 ± 15	88 ± 19	NS
V_1 (liters)	3.4 ± 1.5	4.4 ± 1.1	NS
V_{ss} (liters)	8.4 ± 5	9.2 ± 5.3	NS

* Below assay detection limit. † Difference between 24-h and baseline alteplase antigen concentrations; the remaining p values pertain to differences between antigen and functional activity. $AUC_{0-\infty}$ = partial area under the curve; C_{max} = maximal plasma concentration after 15-mg bolus injection; C_{ss1} and C_{ss2} = steady state plasma concentrations during the 50- and 35-mg infusions, respectively; CL = total clearance; $t_{1/2\alpha}$ and $t_{1/2\beta}$ = plasma half-life; V_1 and V_{ss} = volumes of distribution of the central compartment and at steady state, respectively.

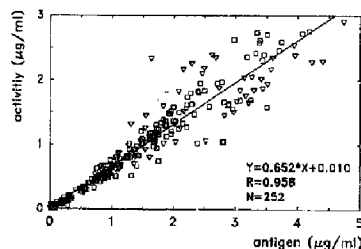


Figure 3. Correlation between alteplase plasma concentrations, measured as antigen (x axis) and functional activity (y axis), in 10 patients with myocardial infarction. Squares and triangles represent data from the "accelerated" and "standard" infusion regimens, respectively.

The mean antigen/activity ratio, derived from the slopes of linear regression plots for individual patients, was 0.65 ± 0.15 and 0.72 ± 0.07 for the accelerated and standard infusion regimens, respectively ($p = NS$). On combining the data for both studies, the graph shown in Figure 3 was obtained, which yielded an overall activity/antigen ratio for 22 patients of 0.65 (95% confidence limits, range 0.62 to 0.68).

Systemic effects. The effects of the accelerated alteplase infusions on hemostasis variables of the study patients are depicted in Figure 4. Mean fibrinogen levels decreased to 61% of baseline by 4 h ($p < 0.001$) but had reverted to 81% at 24 h. The fibrinogen concentration at 4 h was below 0.5 g/liter at 4 h in one patient. Plasminogen and alpha₂-antiplasmin demonstrated a qualitatively similar time dependence, decreasing to 70% and 35% of preinfusion values, respectively, at 2 h but rising to 83% and 88%, respectively, at 24 h. Mean fibrin degradation product values at baseline (0.34 ± 0.19 µg/ml) were slightly above the upper limit of normal, increased to 5.1 ± 2.2 µg/ml at 2 h and were still significantly elevated at 24 h after dosing (0.59 ± 0.16 µg/ml; $p = 0.03$). Fibrinogen degradation products were normal at baseline (0.13 ± 0.19 µg/ml), increased to 1.9 ± 1.05 µg/ml at 2 h but were not significantly different from baseline at 24 h (0.32 ± 0.32 µg/ml).

Discussion

Pharmacokinetics. The principal finding of this clinical pharmacologic study is that during accelerated infusions, initial steady state plasma concentrations of alteplase were significantly higher than during the standard infusion schedule. This was the case whether ELISA or the chromogenic activity assay was used to measure alteplase (Fig. 1). In this small subgroup, 8 of 10 patients exhibited a patent infarct-related coronary artery at 90 min, which is consistent with

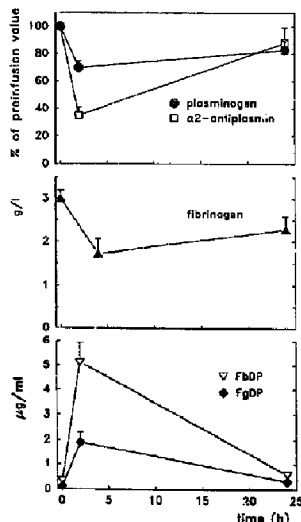


Figure 4. Time course of variables of systemic hemostasis during accelerated infusions of alteplase. FbDP = fibrin degradation products; FgDP = fibrinogen degradation products. Data points are mean values \pm SEM.

the value of 84% for all 217 patients treated with alteplase in the TAPS study (10). Therefore, the mean initial steady state plasma concentrations of 3.1 µg/ml (antigen) or 2.1 µg/ml (activity) are reasonable estimates of the pharmacologic plasma concentrations associated with the increased velocity of coronary thrombolysis previously reported using the "front-loaded" dosage regimen (5,6,10). The steady state concentration after the second, maintenance infusion was only slightly higher after accelerated dosing, and this infusion was of shorter duration than in the standard regimen. Therefore, the elevated initial plasma concentration of alteplase is probably the primary determinant of high early coronary artery patency. The principal function of the maintenance infusions is probably the prevention of reocclusion (18).

Plasma concentrations of free alteplase measured as chromogenic activity were 35% lower than values measured as immunoreactive protein using ELISA, in both the accelerated and the standard infusion regimens (Fig. 1 and 3). This finding confirms and extends data from our earlier studies in healthy volunteers (13,14) and is consistent with a

recent report (19) that demonstrated that the discrepancy is due to complexation of alteplase by plasma protein inhibitors such as alpha₂-antiplasmin. The complexes are partially detectable in the ELISA but not in the activity assay (13). A novel feature of the present study is that good two-compartment pharmacokinetic model fits could be obtained using plasma concentration-time data from both the activity assay and ELISA in patients with myocardial infarction (Fig. 2); in particular, the half-lives and volumes of distribution derived from either analytical method were equivalent.

Pharmacokinetic variables (Table 1) were generally in good agreement with values reported earlier for the standard infusion regimen (8) or a single 50-mg bolus injection (9). In particular, the short alpha plasma half-life was in the range 3.5 to 4.8 min for all three studies; however, alteplase clearance after accelerated infusions was significantly higher than during the standard regimen (8) even though the same assays were used and the patients exhibited similar baseline characteristics in both studies. Therefore, other yet undetermined factors that influence the elimination of alteplase, which occurs primarily through specific uptake in the liver (20), must be responsible for the difference in clearance.

Fibrin specificity. The effects of accelerated infusions on hemostasis (Fig. 4) were moderate. The minor decreases in plasminogen and fibrinogen, and the significant residual level of alpha₂-antiplasmin, indicated that the extent of systemic plasmin generation was only limited. Insofar as bleeding events are correlated with fibrinogenolysis (21), the data provide support for the safety of this regimen. The fibrin specificity of alteplase was underscored by the almost three-fold higher maximal levels of fibrin; degradation as opposed to fibrinogen degradation products. The results are highly comparable with the systemic effects observed after the standard infusion regimen (8). Fibrinogen degradation products and alteplase antigen were significantly elevated at 24 h with respect to baseline, which supports the hypothesis that alteplase exerts prolonged pharmacologic effects despite its short circulatory half-life (22).

Conclusions. The improved efficacy of the "front-loaded" infusion scheme for thrombolytic therapy by alteplase in myocardial infarction is fully rationalized by the pharmacokinetic data obtained in this study and is attained without sacrificing fibrin specificity.

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